PATENT APPLICATION
Mo-6761
LeA 35,018

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICATION OF	)
	)
RUTH MEISSNER ET AL	)
	)
SERIAL NUMBER: TO BE ASSIGNED	)
	)
FILED: HEREWITH	)
	)
TITLE: PLANT PHOSPHOMEVALONATE KINASES	)

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

Upon the granting of a serial number and filing date and prior to the examination of the subject application, kindly amend the application as follows:

IN THE SPECIFICATION:

On page 1, before the paragraph beginning on line 4 with the phrase "The invention relates", please insert the following heading, underlined and centered on the page:

--TECHNICAL FIELD OF THE INVENTION--

On page 1, before the paragraph beginning on line 10 with the phrase "Unwanted plant growth", please insert the following heading, underlined and centered on the page:

--BACKGROUND OF THE INVENTION--.

Please amend the paragraph beginning on page 1, line 7 and ending on page 2, line 7, as follows:

--Isopentyl diphosphate (IPP) is the branching point from which the widest range of isoprenoids are formed. The production of IPP is therefore a critical point in plant metabolism. In plants, IPP is produced via two different metabolic pathways in different compartments. In the endoplasmic reticulum (ER) and in the cytosol, IPP synthesis proceeds via the classic acetate/mevalonate metabolic pathway as it also proceeds in the animal organism. In contrast, IPP is synthesized in chloroplasts via the alternative glyceraldehyde phosphate/pyruvate metabolic pathway. Both metabolic pathways are essential since various isoprenoid metabolites are formed in the different compartments. Moreover, the degree to which the two metabolic pathways are autonomous or to which an exchange of metabolites takes place between the compartments has not been elucidated as yet (Heintze et al., 1990, Kleinig, 1989). (See References section below for full citation to these and other references referred to herein).--

On page 2, before the paragraph beginning on line 18 with the phrase "Within the context", please insert the following heading, underlined and centered on the page:

--SUMMARY OF THE INVENTION--.

Please amend the paragraph beginning on page 2, line 24 and ending on page 3, line 10 as follows:

--The homology between the *Saccharomyces cerevisiae* PMVK (= ERG8) and the cDNA isolated from *A. thaliana* amounts to 44% similarity or 35% identity (see Fig. 1, Bestfit with Wisconsin Package Version 10.1). (ERG8 is the name of the gene encoding phosphomevalonate kinase in yeast (*S cerevisiae*)). This corresponds for example to the homology between the *Saccharomyces cerevisiae* mevalonate kinase and the *Arabidopsis thaliana* mevalonate kinase with a similarity of 45% and an identity of 35%. The function was detected for the *Arabidopsis thaliana* mevalonate kinase by complementation of the corresponding mutant from *Saccharomyces cerevisiae*. Moreover, the cDNA isolated within the context of the present invention shows 69% identity with a partial PMVK sequence from *Pinus radiata* in accordance with SEQ ID NO:5, which is of interest for modifying the isoprenoid content, isoprenoid composition and isoprenoid metabolism of plants (WO 00/36 081). Further partial cDNAs from plants (*Medicago trunculata,* Accession Number AA660847, see SEQ ID NO:3 and *Gossypium hirsutum*, Accession Number AI727861, see SEQ ID NO:4) have been isolated as putative PMVKs. Various Arabidopsis spp. sequences (ESTs and genomic sequences) which correspond to the PMVK sequence isolated herein or to parts thereof can be found in databases from various sequencing projects, however, no information is given on the function or importance of these sequences or sequence fragments.--

On page 3, after the paragraph ending on line 28 with the phrase "fragments thereof." Please insert the following:

--BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a determination of the homology between the *A. thaliana* phosphomevalonate kinase according to the invention in accordance with SEQ ID NO:2 and the known *S. cerevisiae* phosphomevalonate kinase (BESTFIT) by means of Bestfit (Wisconsin Package Version 10.1 (GCG)). The similarity is 44% and the identity 35%.

SEQ ID NO:1 Nucleic acid sequence encoding *A. thaliana* phosphomevalonate kinase.

SEQ ID NO:2 Amino acid sequence of the *A. thaliana* phosphomevalonate kinase.

SEQ ID NO:3 Nucleic acid fragment from *Medicago trunculata* (putative PMVK) of Accession Number AA 660847.

SEQ ID NO:4 Nucleic acid fragment from *Gossypium hirsutum* (putative PMVK) of Accession Number AI 727861.

SEQ ID NO:5 Nucleic acid fragment from *Pinus radiata* (encoding PMVK in accordance with WO 00/36081).--

On page 3, before the paragraph beginning on line 30 with the phrase "The nucleic acids", please insert the following heading, underlined and centered on the page:

--DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS OF THE INVENTION--

On page 18, as the first line before the phrase "Example 1", please insert the following heading, underlined and centered on the page:

--EXAMPLES--.

Please delete on page 20, the text beginning with line 10 and its phrase "Figures and sequence listing" through, and ending with, line 30 and the phrase "Number AA 660847."

Please delete all of the text on page 21, which extends from line 1 and the phrase "SEQ ID NO:4" through line 9 and the phrase "WO 00/36081").

On page 23, after the paragraph ending on line 11 with its phrase "432" please insert the following new paragraph:

-- Although the invention has been described in detail in the foregoing for the purpose of illustration, it is to be understood that such detail is solely for that purpose and that variations can be made therein by those skilled in the art without departing from the spirit and scope of the invention except as it may be limited by the claims. --

IN THE CLAIMS:

Please amend the claims as follows. Please delete Claims 2-24 and replace them with the following new Claims 25 - 58.

--25. An isolated nucleic acid encoding a plant phosphomevalonate kinase, selected from the group consisting of:

(a) the sequence in accordance with SEQ ID NO: 1,

(b) sequences which encode a polypeptide which encompasses the amino acid sequence in accordance with SEQ ID NO: 2,

(c) partial sequences of the sequences defined under (a) or (b) which have a length of at least 14 base pairs,

(d) sequences which hybridize with the sequences defined under (a) or (b) at a hybridization temperature of 35-52°C,

(e) sequences which have at least 70% identity with the sequences defined under (a) or (b),

(f) sequences which are complementary to the sequences defined under a) or b), and

(g) sequences which, owing to the degeneracy of the genetic code, encode the same amino acid sequence as the sequences defined under a) to e).

26. An isolated nucleic acid according to Claim 25, selected from the group consisting of:

(a) the sequence in accordance with SEQ ID NO: 1,

(b) sequences which encode a polypeptide which encompasses the amino acid sequence in accordance with SEQ ID NO: 2,

(c) sequences which are complementary to the sequences defined under a) or b), and

(d) sequences which, owing to the degeneracy of the genetic code, encode the same amino acid sequence as the sequences defined under a) to c).

27. A DNA construct encompassing a nucleic acid according to Claim 25 and a heterologous promoter.

28. A DNA construct encompassing a nucleic acid according to Claim 26 and a heterologous promoter.

29. A vector encompassing a nucleic acid according to Claim 25.

30. A vector encompassing a nucleic acid according to Claim 26.

31. A vector encompassing a DNA construct according to Claim 27.

32. A vector encompassing a DNA construct according to Claim 29.

33. A vector according to Claim 29, characterized in that the nucleic acid is linked functionally to regulatory sequences which ensure expression of the nucleic acid in pro- or eukaryotic cells.

34. Vector according to Claim 30, characterized in that the nucleic acid is linked functionally to regulatory sequences which ensure expression of the nucleic acid in pro- or eukaryotic cells.

35. A host cell comprising a nucleic acid according to Claim 25.

36. A host cell comprising a DNA construct according to Claim 27.

37. A host cell comprising a vector according to Claim 29.

38. A host cell according to Claim 35, characterized in that it is a prokaryotic cell.

39. A host cell according to Claim 35, characterized in that it is a eukaryotic cell.

40. An isolated polypeptide with the biological activity of a phosphomevalonate kinase which is encoded by a nucleic acid according Claim 25.

41. An isolated polypeptide with the biological activity of a phosphomevalonate kinase which is encoded by a nucleic acid according Claim 26.

42. An isolated polypeptide with the biological activity of a phosphomevalonate kinase which encompasses an amino acid sequence with at least 70% identity with the sequence in accordance with SEQ ID NO: 2.

43. An antibody which binds specifically to a polypeptide according to Claim 40.

44. An antibody which binds specifically to a polypeptide according to Claim 41.

45. A method of generating a nucleic acid according to Claim 25, comprising a step selected from:

(a) chemically synthesizing the nucleic acid,

(b) chemical synthesizing oligonucleotides, labeling of the oligonucleotides, hybridizing of the oligonucleotides with DNA of a genomic or cDNA library which had been generated starting from genomic DNA or mRNA from plant cells, selecting positive clones, and isolating the hybridizing DNA from positive clones, and

(c) chemical synthesizing oligonucleotides and amplifying the target DNA using PCR.

46. A method of generating a polypeptide with the biological activity of a phosphomevalonate kinase which is encoded by a nucleic acid according to Claim 1, comprising:

(a) culturing a host cell comprising a nucleic acid according to Claim 25 under conditions which ensure expression of the nucleic acid according to Claim 25, and

(b) obtaining the polypeptide from the host cell or the culture medium

47. A method of generating a polypeptide with the biological activity of a phosphomevalonate kinase which is encoded by a nucleic acid according to Claim 25, comprising

(a) expressing a nucleic acid according to Claim 25 in an *in-vitro* system, and

(b) obtaining the polypeptide from the *in-vitro* system.

48. A method of finding a chemical compound which binds to a polypeptide with the biological activity of a phosphomevalonate kinase which is encoded by a nucleic acid according to Claim 25 and/or modulates the activity of this polypeptide, encompassing the following steps:

(a) contacting a host cell comprising a nucleic acid according to Claim 25 or a polypeptide with the biological activity of a phosphomevalonate kinase which is encoded by a nucleic acid according to Claim 25 with a chemical compound or a mixture of chemical compounds under conditions which permit the interaction of a chemical compound with the polypeptide, and

(b) comparing the biological activity of the polypeptide in the presence of a chemical compound or a mixture of chemical compounds with the biological activity of the polypeptide in the absence of a chemical compound or a mixture of chemical compounds, and

(C) determining the chemical compound which specifically binds to the polypeptide and/or specifically modulates the biological activity of the polypeptide.

49. A method of finding a compound which modifies the expression of polypeptide with the biological activity of a phosphomevalonate kinase which is encoded by a nucleic acid according to Claim 25, comprising:

(a) contacting a host cell comprising a nucleic acid according to Claim 25 with a chemical compound or a mixture of chemical compounds,

(b) determining the polypeptide concentration, and

(c) determining the compound which specifically affects the expression of the polypeptide.

50. A modulator which is identified by a method according to Claim 48.

51. A modulators which is identified by a method according to Claim 48.

52. A herbicidally active substance which is found by a method according to Claim 48.

53. A herbicidally active substance which is found by a method according to Claim 49.

54. An isolated nucleic acid encoding a plant phosphomevalonate kinase, with the exception of the nucleic acid fragments in accordance with SEQ ID NO: 3, 4 and 5.

55. An isolated nucleic acid according to Claim 54, wherein the isolated nucleic acid encodes an *A. thaliana* phosphomevalonate kinase.

56. An isolated nucleic acid according to Claim 54, wherein the isolated nucleic acid is a single-stranded or double-stranded DNA or RNA.

57. An isolated nucleic acid according to Claim 54, wherein the isolated nucleic acid is a fragment of genomic DNA or cDNA.

58. An isolated nucleic acid according to Claim 54, wherein the isolated nucleic acid is derived from *A. thaliana*.--

REMARKS

This amendment is made to place the claims in conformance with U.S. patent practice and to claim the present invention in more varying scope. Claims 2-24 have been cancelled and replaced with new Claims 25- 58. Claim 1 has been permitted to remain only to ensure copendency, but will be cancelled in response to the first Office Action on the merits in the case.

This amendment is not in derogation of any prior art, and Applicant respectfully asserts that it is entitled to the claims as amended and any equivalents thereof.

Respectfully submitted,

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/jme/RJH0017

Version Marked to Show Changes

IN THE SPECIFICATION:

As explicitly set forth in **37 C.F.R. Section 1.121(b)(1)(iii), last sentence**, a marked up version does not have to be supplied for an added paragraph or a deleted paragraph as it is sufficient to state that a particular paragraph has been added, or cancelled, and this has been so stated in the Amendment.

In particular, in this case, section headings have been added in several places in the specification, (e.g. Technical Field of the Invention, Background of the Invention, Summary of the Invention, Brief Description of the Drawings, etc). Also, paragraphs have been added to provide a brief description of the drawings under the corresponding heading. Redundant paragraphs also briefly describing the drawings have been removed from the end of the specification. Only the following two paragraphs have been amended:

Please amend the paragraph beginning on page 1, line 7 and ending on page 2, line 7, as follows:

--Isopentyl diphosphate (IPP) is the branching point from which the widest range of isoprenoids are formed. The production of IPP is therefore a critical point in plant metabolism. In plants, IPP is produced via two different metabolic pathways in different compartments. In the endoplasmic reticulum (ER) and in the cytosol, IPP synthesis proceeds via the classic acetate/mevalonate metabolic pathway as it also proceeds in the animal organism. In contrast, IPP is synthesized in chloroplasts via the alternative glyceraldehyde phosphate/pyruvate metabolic pathway. Both metabolic pathways are essential since various isoprenoid metabolites are formed in the different compartments. Moreover, the degree to which the two metabolic pathways are autonomous or to which an exchange of metabolites takes place between the compartments has not been elucidated as yet (Heintze et al., 1990,

Kleinig, 1989). (See References section below for full citation to these and other references referred to herein).--

Please amend the paragraph beginning on page 2, line 24 and ending on page 3 ,line 10 as follows:

The homology between the *Saccharomyces cerevisiae* PMVK (= ERG8) and the cDNA isolated from *A. thaliana* amounts to 44% similarity or 35% identity (see Fig. 1, Bestfit with Wisconsin Package Version 10.1). (ERG8 is the name of the gene encoding phosphomevalonate kinase in yeast (*S cerevisiae*)). This corresponds for example to the homology between the *Saccharomyces cerevisiae* mevalonate kinase and the *Arabidopsis thaliana* mevalonate kinase with a similarity of 45% and an identity of 35%. The function was detected for the *Arabidopsis thaliana* mevalonate kinase by complementation of the corresponding mutant from *Saccharomyces cerevisiae*. Moreover, the cDNA isolated within the context of the present invention shows 69% identity with a partial PMVK sequence from *Pinus radiata* in accordance with SEQ ID NO:5, which is of interest for modifying the isoprenoid content, isoprenoid composition and isoprenoid metabolism of plants (WO 00/36 081). Further partial cDNAs from plants (*Medicago trunculata,* Accession Number AA660847, see SEQ ID NO:3 and *Gossypium hirsutum*, Accession Number AI727861, see SEQ ID NO:4) have been isolated as putative PMVKs. Various Arabidopsis spp. sequences (ESTs and genomic sequences) which correspond to the PMVK sequence isolated herein or to parts thereof can be found in databases from various sequencing projects, however, no information is given on the function or importance of these sequences or sequence fragments.

IN THE CLAIMS:

As explicitly set forth in **37 C.F.R. Section 1.121(c)(1)(ii), last sentence**, a marked up version does not have to be supplied for an added claim or a cancelled claim as it is sufficient to state that a particular claim has been added, or cancelled, and this has been so stated in the Amendment.

In particular, in this case, Claims 2-24 have been cancelled, and Claims 25 - 58 have been newly added.